Laboratory methods for diagnosis and management of plasma cell neoplasms

MLS Symposium, Auburn University-Montgomery, March 2023

Presented by: Andrew N. Young, MD, PhD

Quest Diagnostics®

POWERING AFFORDABLE CARE SINCE 1967

Disclosures

Andrew N. Young, MD, PhD, Regional Medical Director, Quest Diagnostics

- Andrew N. Young is an employee of and owns stock in Quest Diagnostics.

Learning objectives

Attendees will become familiar with:

- The diagnostic classification of plasma cell neoplasms and the role of clinical-laboratory correlation
- The primary laboratory methods for diagnosis and monitoring of disease
- Emerging laboratory methods to support the diagnosis, prognosis, and selection of therapy

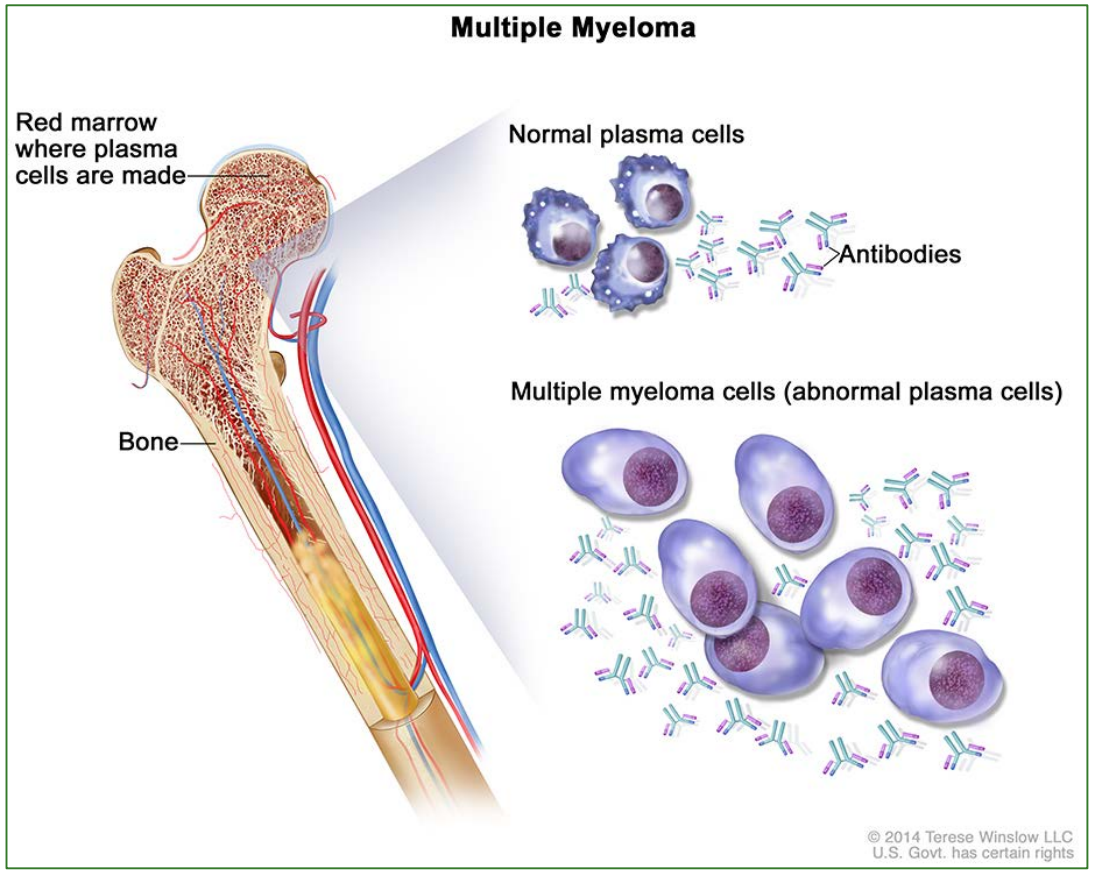
Multiple myeloma

Also termed plasma cell myeloma

- A malignant **plasma cell neoplasm**
  - Clonal plasma cells accumulate in bone marrow and produce monoclonal immunoglobulin (**M-Protein**)
- Complicated by end-organ damage
  - Hyper**C**alcemia
  - **R**enal dysfunction
  - **A**nemia
  - **B**one destruction

←**"CRAB"** findings

- Arises from precursor neoplasm
  - Monoclonal gammopathy of undetermined significance (**MGUS**)
- Progresses through further clonal evolution



Centers for Disease Control and Prevention. Myeloma. Updated Jul 6, 2022. Accessed Mar 24, 2023. https://www.cdc.gov/cancer/myeloma/index.htm

Quest Diagnostics

Multiple myeloma

Clinical characteristics

- Median age at diagnosis: 65-70 years
- Risk factors
  - Obesity, inflammation, exposure to organic solvents
  - Possible genetic variants
- Therapy
  - Proteasome inhibitors, immunomodulatory agents
  - Antibodies against plasma cell surface molecules
  - Stem cell transplantation
  - CAR-T cells, bispecific T cell engagers
- Median overall survival
  - 10 years if transplant eligible, 4-5 years if not eligible

US myeloma incidence rates 2015-2019
American Cancer Society, CancerStatisticsCenter.org

Incidence by sex	Rate per 100,000
Male	**8.6**
Female	5.9

Incidence by race or ethnicity	Rate per 100,000
Non-Hispanic Black	**14.3**
Native American	8.1
Hispanic	6.8
Non-Hispanic White	6.2
Asian/Pacific Islander	3.9

Classification of plasma cell neoplasms

International Myeloma Working Group

- **Monoclonal gammopathy of undetermined significance (MGUS):** premalignant precursor condition
  - Serum monoclonal protein <3 g/dL
  - Clonal bone marrow plasma cells <10%
  - Absence of end-organ damage (CRAB findings)

- **Smoldering multiple myeloma (SMM):** higher-risk premalignant precursor condition
  - Serum monoclonal protein ≥3 g/dL or urine monoclonal protein ≥500 mg/24 hour
  - Clonal bone marrow plasma cells 10%-60%
  - Absence of end-organ damage or myeloma-defining events

- **Active multiple myeloma (MM):** malignant condition
  - Clonal bone marrow plasma cells ≥10%
  - Presence of end-organ damage or myeloma-defining events

Classification of plasma cell neoplasms requires correlation of clinical, radiographic, and laboratory data

Rajkumar SV, Dimopoulos MA, Palumbo A, et al. *Lancet Oncol.* 2014;15:e538-e548.

End-organ damage and myeloma-defining events

International Myeloma Working Group

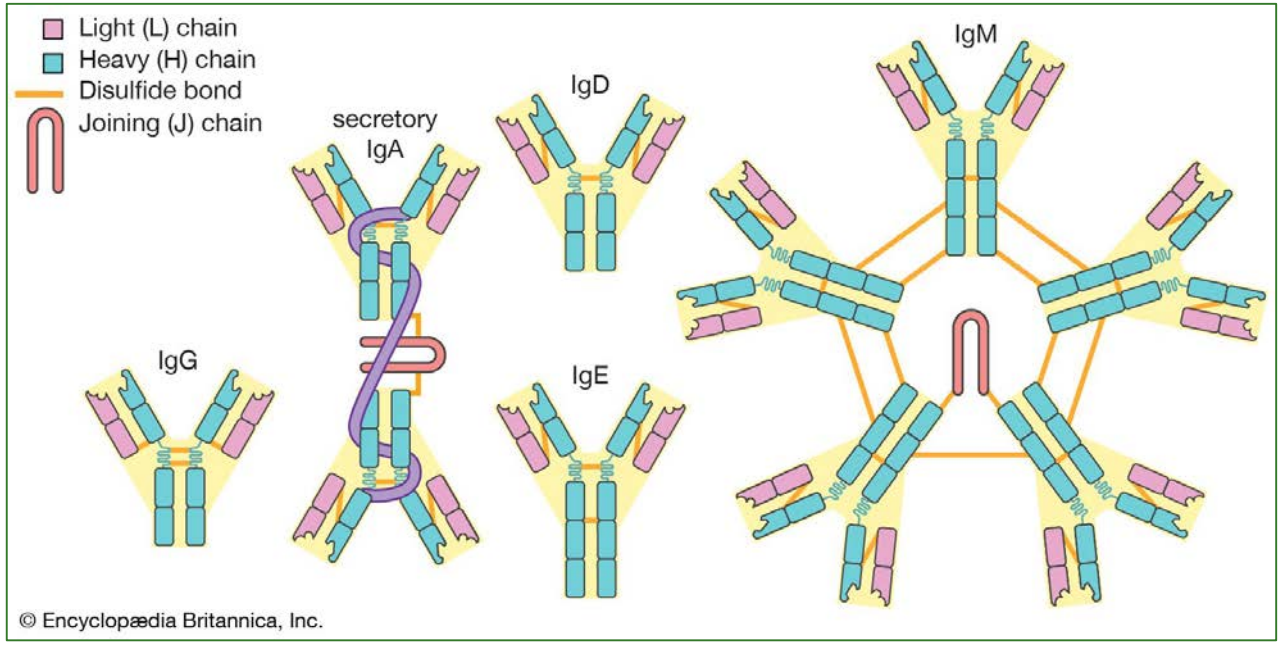
Abnormality	Criteria	Causes
Hypercalcemia	• Serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)	• Osteolytic lesions • Renal insufficiency
Renal insufficiency	• Creatinine clearance <40 mL per minute or serum creatinine >177 mol/L (>2 mg/dL)	• Clonal light chain deposition • Clonal light chain amyloid • Hypercalcemia
Anemia	• Hemoglobin value of >2 g/dL below the lowest limit of normal or <10 g/dL	• Clonal growth in bone marrow • Renal Insufficiency
Bone lesions	• One or more osteolytic lesion on skeletal radiography, CT, or PET/CT	• Osteolytic lesions
Myeloma-defining events	• Clonal bone marrow plasma cells ≥60% • Serum free light chain ratio ≥100, with involved light chain >100 mg/L • More than one focal lesion on MRI that is at least 5 mm or greater in size	• Clonal growth in bone marrow

Rajkumar SV, Dimopoulos MA, Palumbo A, et al. *Lancet Oncol.* 2014;15:e538-e548.

M-protein

Monoclonal immunoglobulin produced by a plasma cell neoplasm

- Biomarker of disease and source of pathology
- Monoclonal IgM is seen primarily in lymphomas (eg, Waldenstrom macroglobulinemia)



Britannica. Immune system. Accessed Mar 24, 2023. https://www.britannica.com/science/immune-system/Classes-of-immunoglobulins

Ig isotype	Frequency	Notes
IgG κ or λ	>50%	Most common finding
IgA κ or λ	~20%	Unfavorable prognosis
IgM κ or λ	Rare	Hyperviscosity
IgD κ or λ	Rare	Unfavorable prognosis
IgE κ or λ	Rare	Unfavorable prognosis
Free κ or λ	~15%	Risk of renal insufficiency
Heavy chain	Rare	Distinct syndromes
Non-secretory	3%	May produce free κ or λ

Recommended laboratory workup

International Myeloma Working Group

- **NOTE:** laboratory and pathology testing done in conjunction with clinical evaluation and imaging studies

- **Routine laboratory testing**
  - Assess blood cells: eg, complete blood count (CBC)
  - Assess kidney function: eg, serum creatinine, creatinine clearance, estimated glomerular filtration gate (eGFR)
  - Assess proteins and other substances in the blood: eg, serum calcium, total protein, beta-2 microglobulin (sβ2M), lactate dehydrogenase (LDH)
  - Assess monoclonal protein
    - Serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE) of blood or urine
    - Quantitative immunoglobulins (QIg) and serum free light chain evaluation

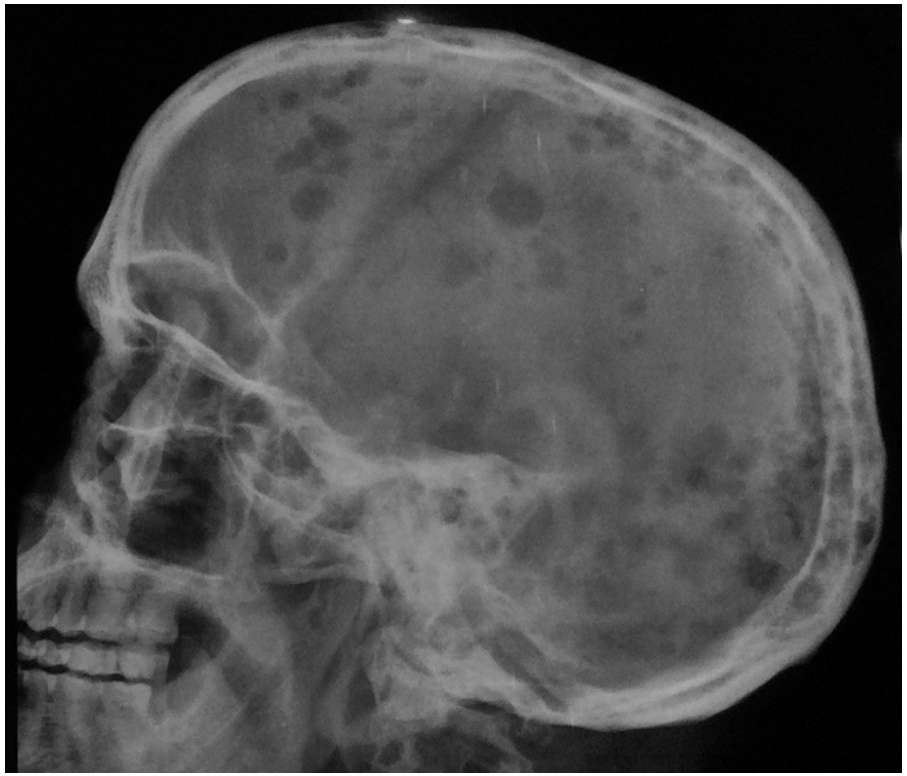
- **Bone marrow testing**
  - Aspirate for Wright-stained cell morphology plus trephine biopsy for H&E-stained histology
  - Testing for cytogenetics, fluorescent in situ hybridization (FISH), and immunophenotyping

Rajkumar SV, Dimopoulos MA, Palumbo A, et al. *Lancet Oncol.* 2014;15:e538-e548.

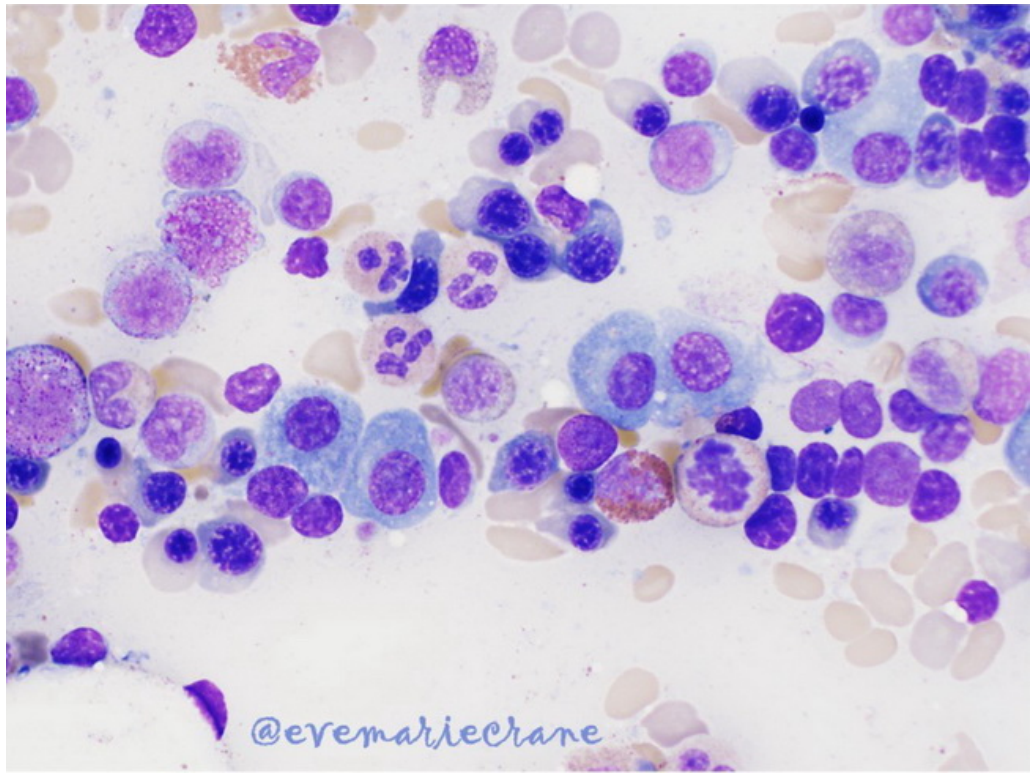
Radiology and bone marrow morphology

Lytic lesions on X-ray; increased atypical plasma cells in bone marrow

Skull X-ray



Wright-stained bone marrow aspirate



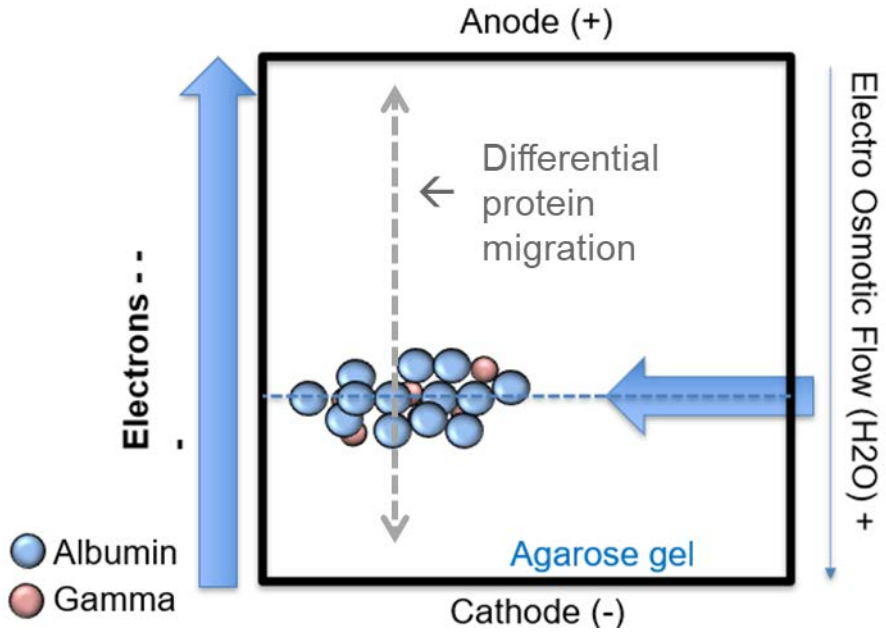
Web Pathology. Myeloma. Accessed Mar 24, 2023.
https://www.webpathology.com/image.asp?n=18&Case=768

Serum protein electrophoresis

Detection and quantification of monoclonal immunoglobulins

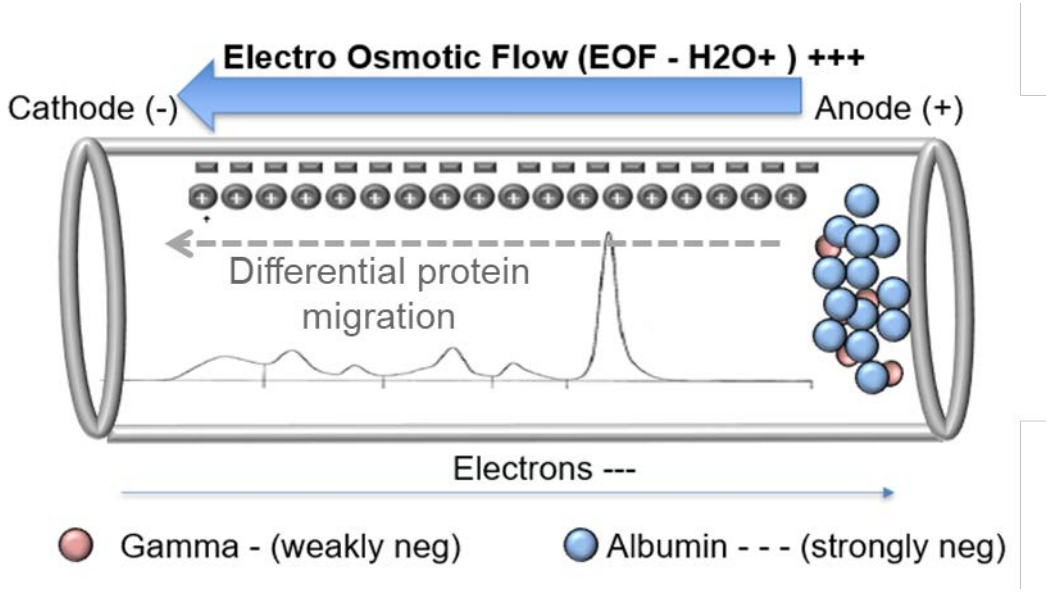
Gel electrophoresis

- Proteins separated in solid medium by electric charge
- Proteins precipitated in gel and stained after migration
- Proteins measured by stain intensity



Capillary electrophoresis

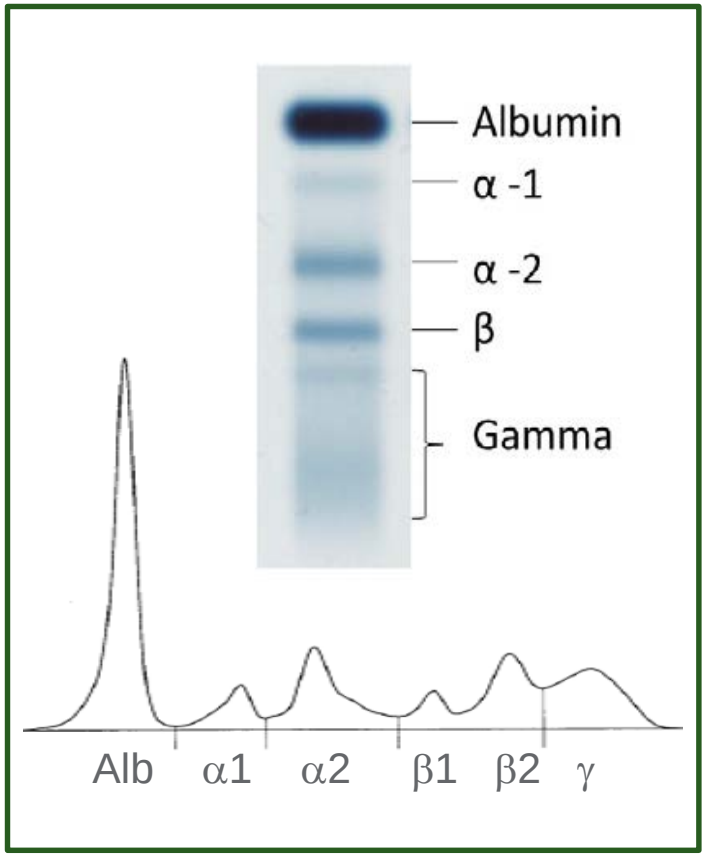
- Proteins separated in liquid medium by electric charge
- Proteins measured by UV absorbance during migration



Diagrams courtesy of Sebia

Serum protein electrophoresis

Components of normal protein fractions

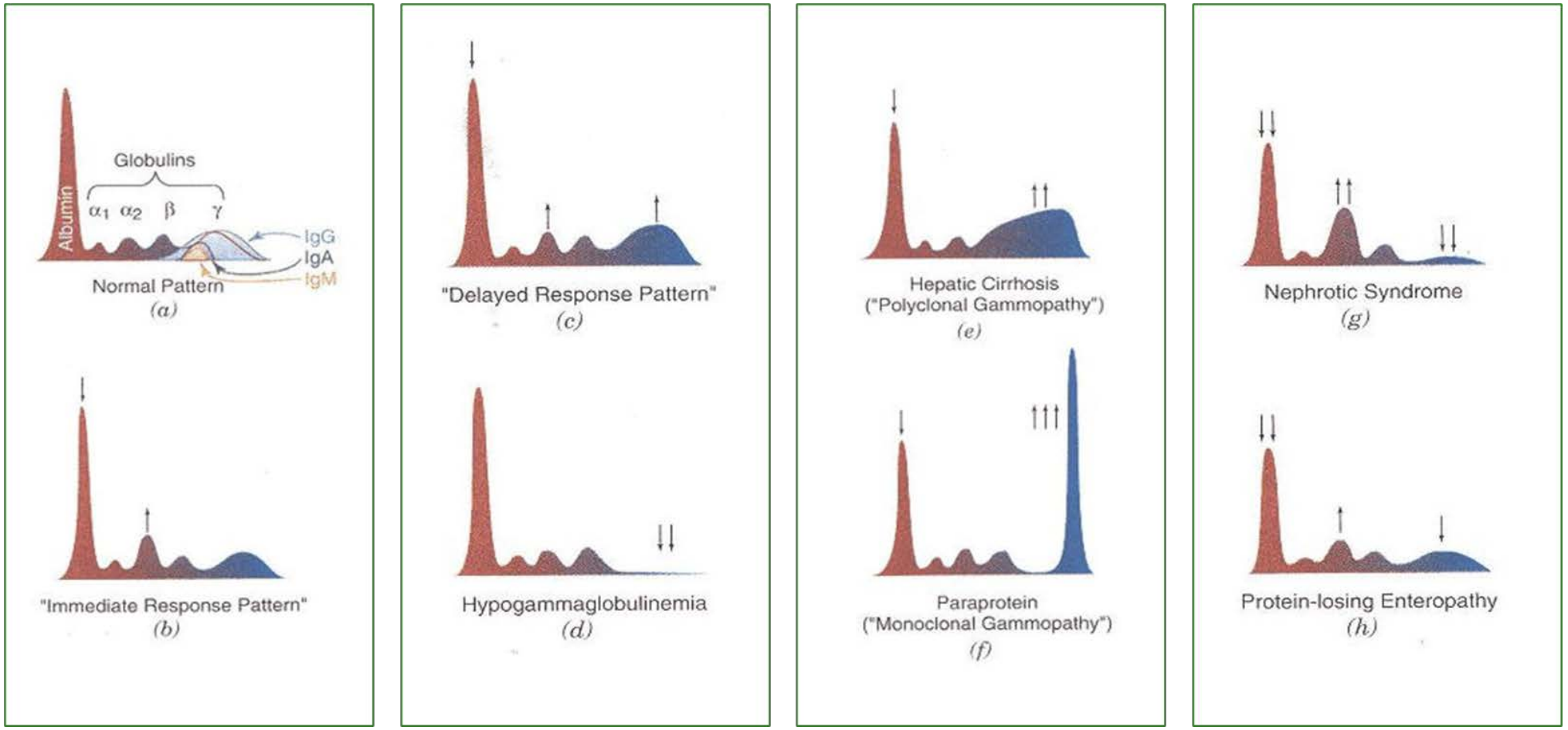


Fraction	Main component(s)	Significance
Alb	• Albumin	• Major serum protein; negative acute phase reactant
Alpha-1	• α1-antitrypsin (A1AT)	• Acute phase reactant; ↓ in inherited A1AT deficiency
Alpha-2	• α2-macroglobulin • Ceruloplasmin • Haptoglobin	• Large protein; ↑ in protein loss (relative retention) • Acute phase reactant; ↓ in Wilson disease • Acute phase reactant; ↓ in hemolysis
Beta-1	• Transferrin • β1-lipoprotein	• Negative acute phase reactant; ↑ in iron deficiency • Large molecule; ↑ in protein loss (relative retention)
Beta-2	• Complement C3 & C4	• ↑ in inflammation; ↓ in immune complex disease
Gamma	• Immunoglobulins	• Polyclonal hump versus monoclonal peak

Morrison T, Booth RA, Hauff K, et al.
Adv Clin Chem. 2019;89:1-58

Serum protein electrophoresis

Interpretation of patterns

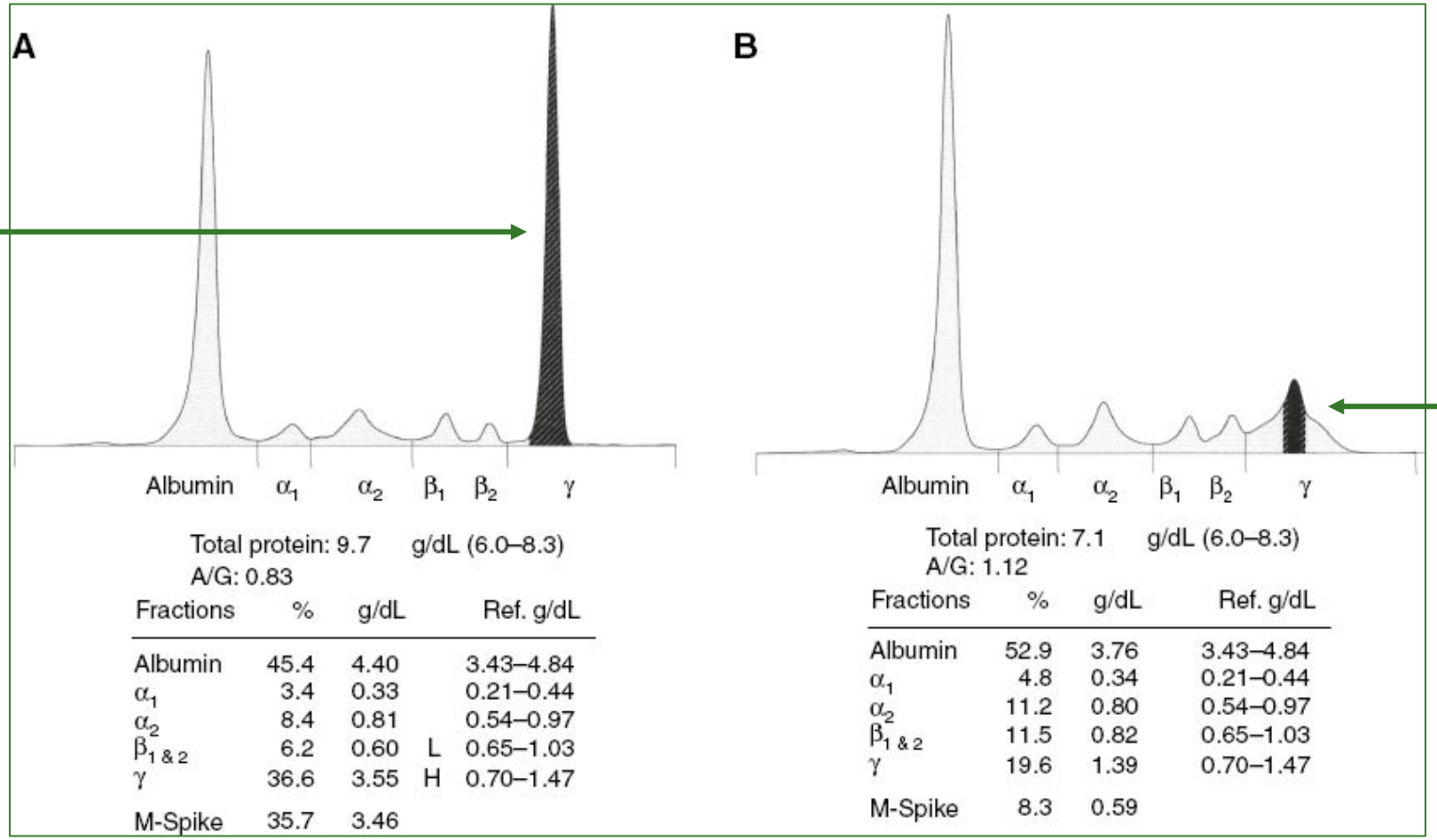


BCIT News. Interpreting serum protein electrophoresis (SPE) patterns. Accessed Mar 24, 2023. https://commons.bcit.ca/news/2018/11/interpreting-serum-protein-electrophoresis-patterns/

Serum protein electrophoresis

Interpretation of patterns

- Large M-protein
- Suppression of polyclonal gamma globulins
- Easier to quantify M-protein

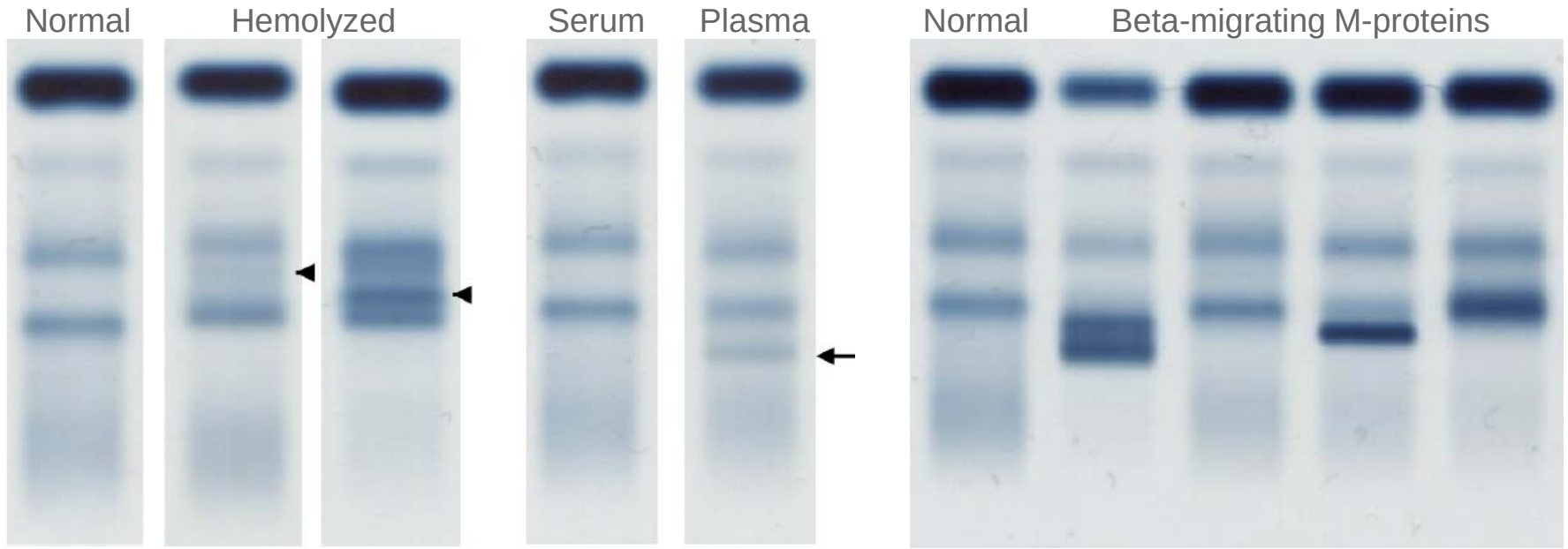


- Small M-protein
- Persistence of polyclonal gamma globulins
- More difficult to quantify M-protein

Keren DF and Schroeder L. *Clin Chem Lab Med.* 2016;54:947–961

Serum protein electrophoresis

Challenges in interpretation: interference and co-migrating M-proteins



Hemoglobin-haptoglobin complex in α2-β1 zone

Fibrinogen band in β2-γ zone

M-proteins that co-migrate with normal β-globulins are difficult to quantify

Interventions

Hemolysis

- Visually examine specimen
- Immunotype

Fibrinogen

- Treat with thrombin
- Immunotype

Co-migrating M-protein

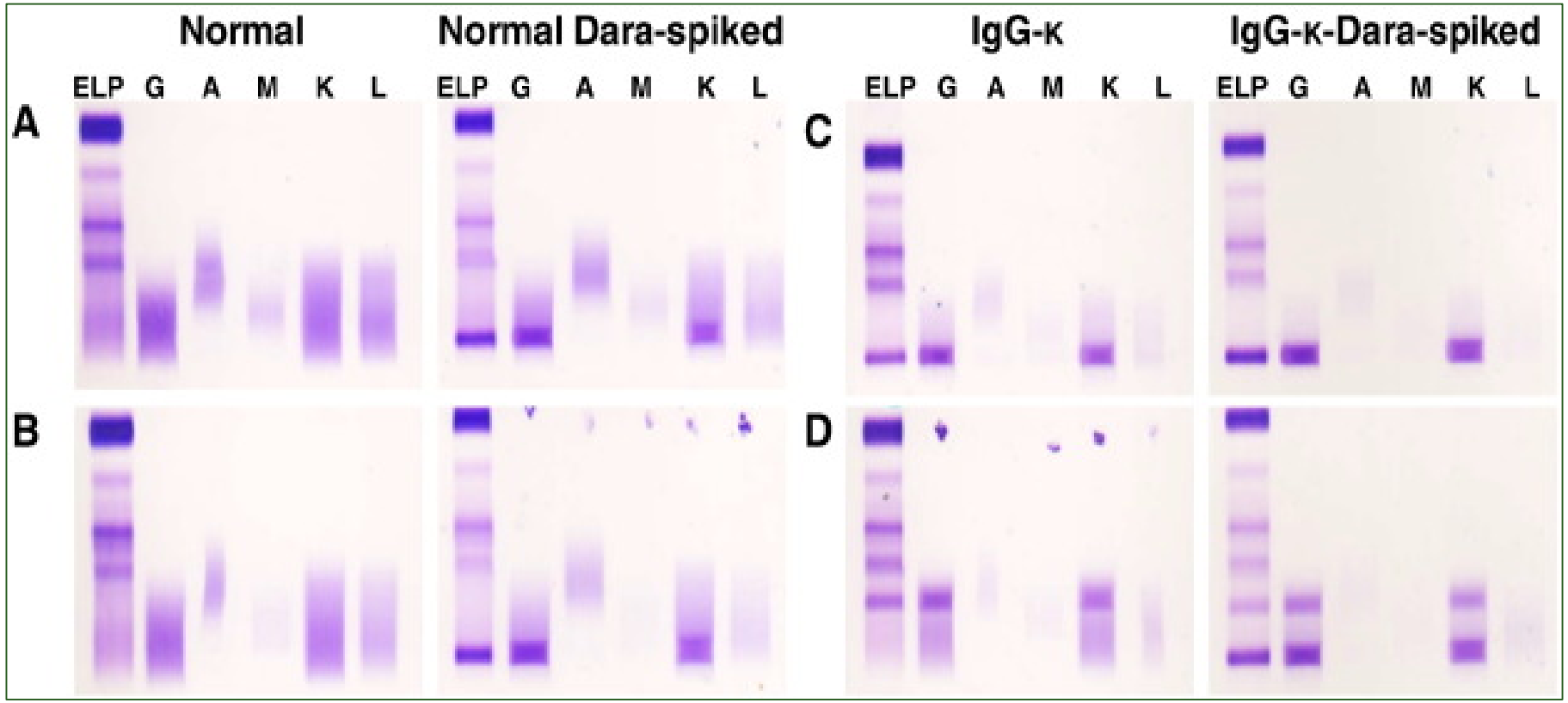
- Immunotype
- Quantify by alternate method

Morrison T, Booth RA, Hauff K, et al. *Adv Clin Chem.* 2019;89:1-58

Serum protein electrophoresis

Challenges in interpretation

Novel monoclonal antibody therapy can mimic M-proteins

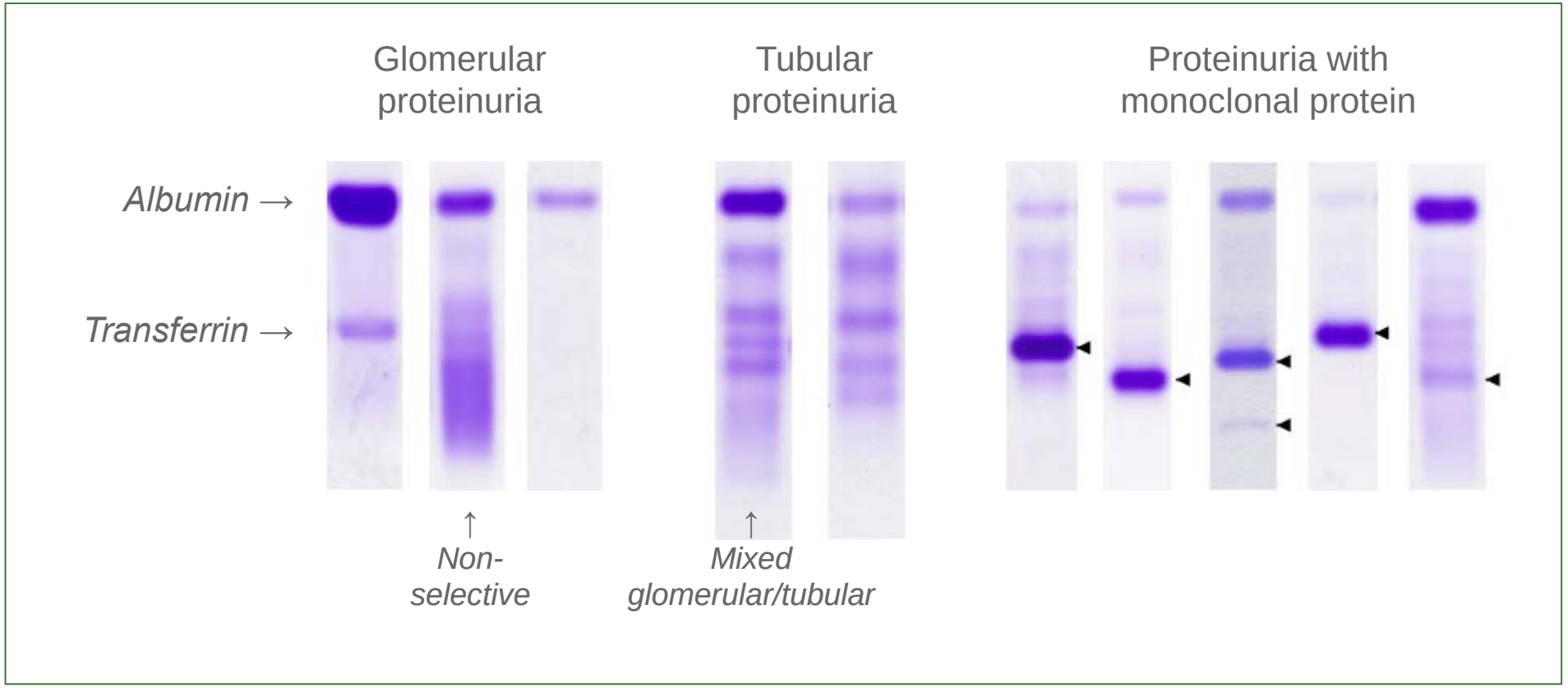


Rosenberg AS, Bainbridge S, Pahwa R, et al. *Clin Biochem.* 2016;49:1202-1204

Quest Diagnostics

Urine protein electrophoresis

Detection and quantification of monoclonal immunoglobulins

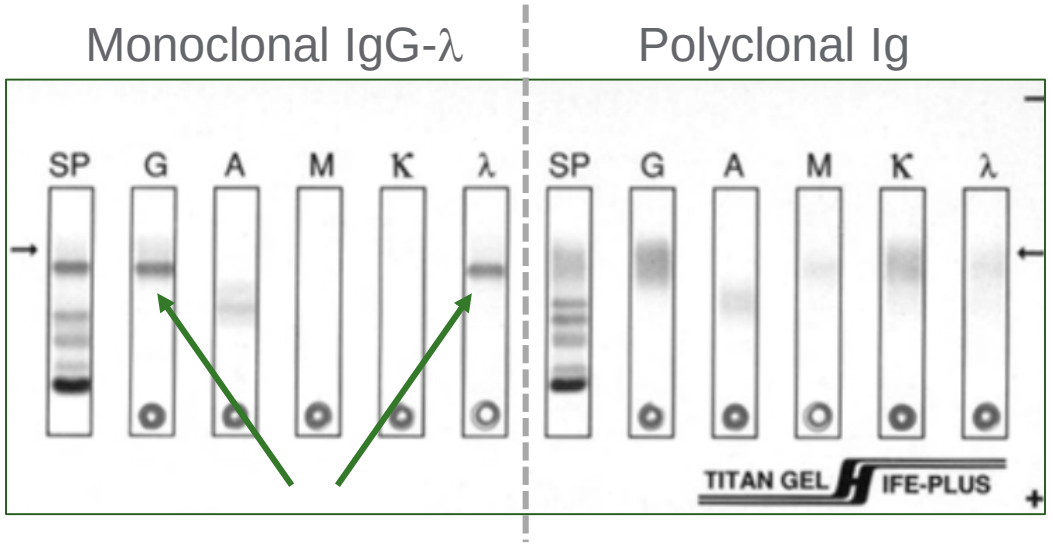


Morrison T, Booth RA, Hauff K, et al. *Adv Clin Chem.* 2019;89:1-58

Immunotyping: immunofixation electrophoresis

Characterization of heavy and/or light chains in M-proteins

- Proteins are separated in agarose gel.
- Control lane is fixed (precipitated).
- Other lanes are treated with specific antisera.
  - Antisera are specific for IgG, IgA, IgM, κ, and λ.
  - Antisera for IgD, IgE are included when indicated.
- Antisera form immune complexes with immunoglobulins.
  - Complexes are large and become “fixed” in the gel.
- Unfixed proteins are washed from gel and remaining fixed proteins are stained.
- Immunofixation is a non-quantitative method.

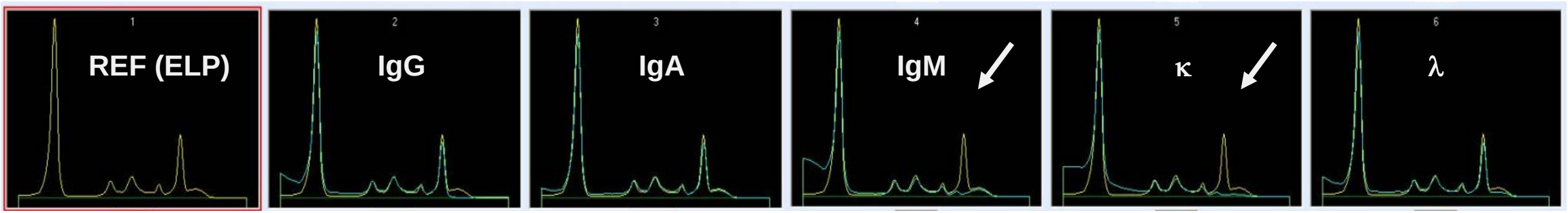


Helena Laboratories. Titan gel Immunofix-Plus procedure. Accessed Mar 24, 2023. https://www.helena.com/Procedures/Pro109Rev7.pdf

Immunotyping: immunosubtraction

Capillary electrophoresis-based alternative to immunofixation electrophoresis

- Serum or urine proteins are mixed with specific antisera prior to capillary electrophoresis.
  - Immunoglobulins in specimen react specifically with their corresponding antiserum.
  - Immunoglobulin/antiserum complexes do not migrate significantly during capillary electrophoresis.
  - After electrophoresis, each antiserum pattern (IgG, IgA, IgM, κ, λ) is compared with untreated reference pattern.
- Disappearance of abnormal peak in antiserum-treated pattern indicates a monoclonal protein.
- Like immunofixation electrophoresis, immunosubtraction is a non-quantitative method.

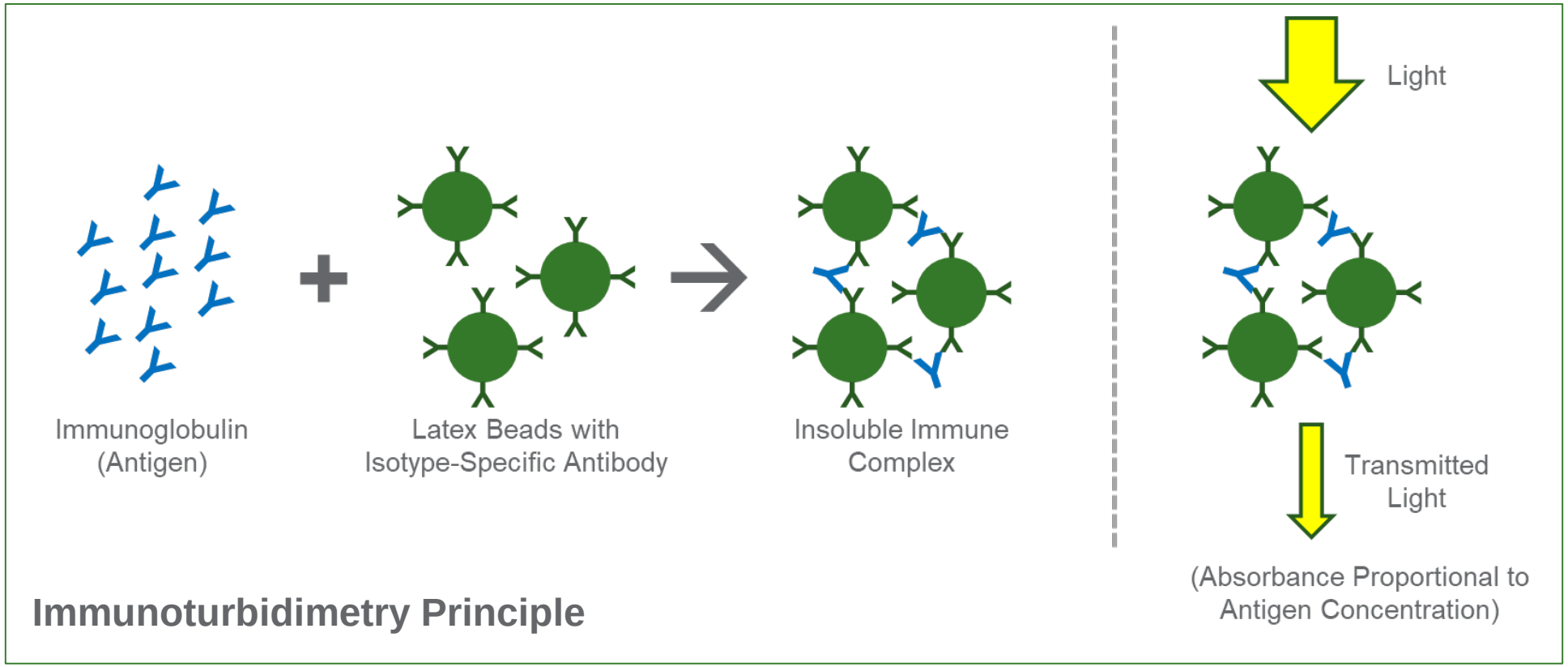


Images courtesy of Sebia

Quantitative immunoglobulin & free light chain analysis

Complementary methods to quantify M-protein

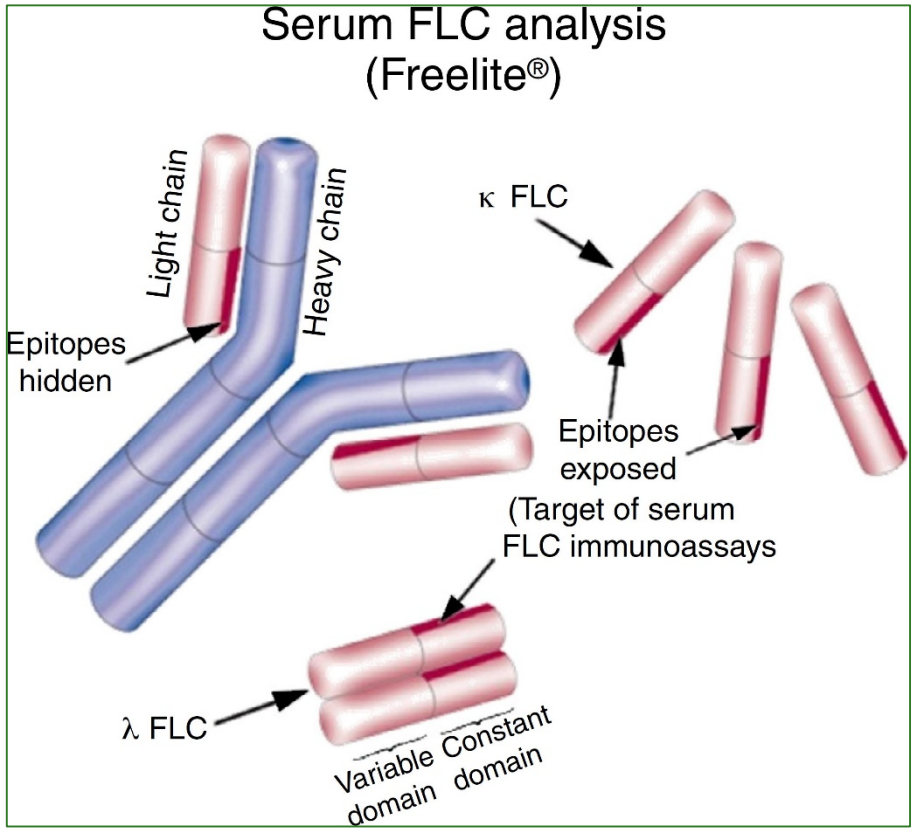
- Immunometric assays such as immunoturbidimetry and nephelometry



Serum free light chain analysis

Immunoturbidimetric assay

- Directed against light chain epitopes that are hidden in intact immunoglobulins
  - Free κ and λ concentrations are measured
  - κ/λ ratio is calculated (abnormal high or low ratio is suggestive of clonality)
- Several important applications
  - Diagnosis of non-secretory myeloma
  - Monitoring course of disease and therapy
  - Potential prognostication for MGUS



Hungria VTM, Allen S, Kampanis P, et al.
Rev Bras Hematol Hemoter. 2016;38:37-43

Laboratory diagnosis of myeloma

Utility of protein electrophoresis, immunotyping, and free light chain analysis

- Sensitivity for plasma cell neoplasms of individual tests compared to test panels
  - Test panels have higher sensitivity because they account for light chain-only and oligo/non-secretory neoplasms

Diagnosis	SPEP SIFE SFLC UIFE	SPEP SIFE UIFE	SPEP SIFE SFLC	SPEP SFLC	SIFE	SPEP	SFLC
Multiple myeloma (MM)	100.0	98.7	100.0	100.0	94.4	87.6	96.8
Smoldering multiple myeloma (SMM)	100.0	100.0	100.0	99.5	98.4	94.2	81.2
Monoclonal gammopathy of undetermined significance (MGUS)	100.0	100.0	97.1	88.7	92.8	81.9	42.4

(S)=Serum; (U)=Urine; PEP=Protein electrophoresis; IFE=Immunofixation; FLC=Free light chain analysis

Katzmann JA. *Clin Biochem Rev.* 2009;30:105-11

Monitoring of disease in myeloma

Utility of protein electrophoresis, immunotyping, and free light chain analysis

Response category	Criteria of the International Myeloma Working Group
Sustained complete response (sCR)	Complete response as defined below plus • Normal FLC ratio and • Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
Complete response (CR)	• Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and • <5% plasma cells in bone marrow
Very good partial response (VGPR)	• Serum and urine M-protein detectable by immunofixation but not on electrophoresis or • >90% reduction in serum M-protein plus urine M-protein level <100 mg/24 h
Partial response (PR)	• 50% reduction of serum M-protein and • >90% reduction in 24 hours urinary M-protein, or • >50% reduction in the difference between involved and uninvolved FLC levels
Progressive disease	Increase of >25% from lowest response value in any one or more of the following: • Serum M-protein and/or • Urine M-protein and/or • Difference between involved and uninvolved FLC levels (if M-Protein undetectable) • Bone marrow plasma cell percentage; the absolute percentage must be >10%

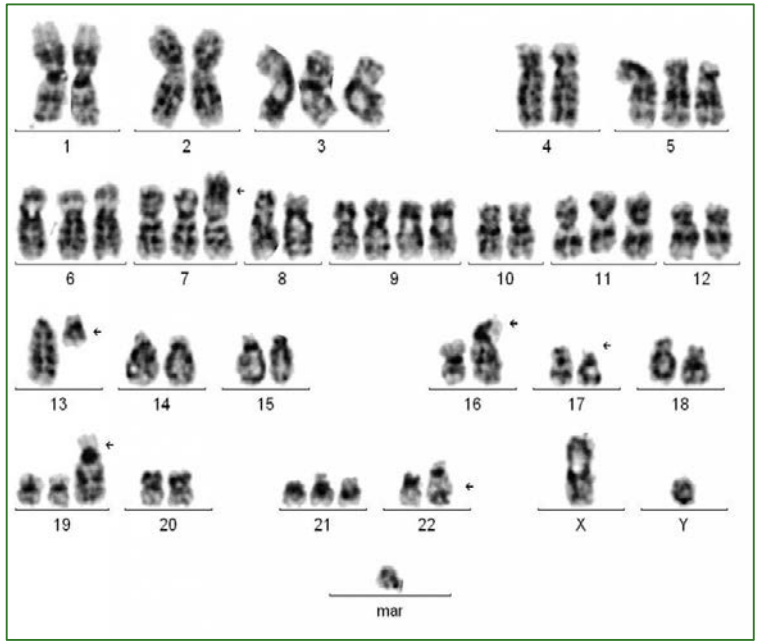
Durie BGM, Harousseau J-L, Miguel JS et al. *Leukemia.* 2006;20:1467–1473

Cytogenetics

Routine chromosome analysis and fluorescence in situ hybridization (FISH)

Chromosome Analysis

Atlas del GECH - https://atlas.gechem.org/

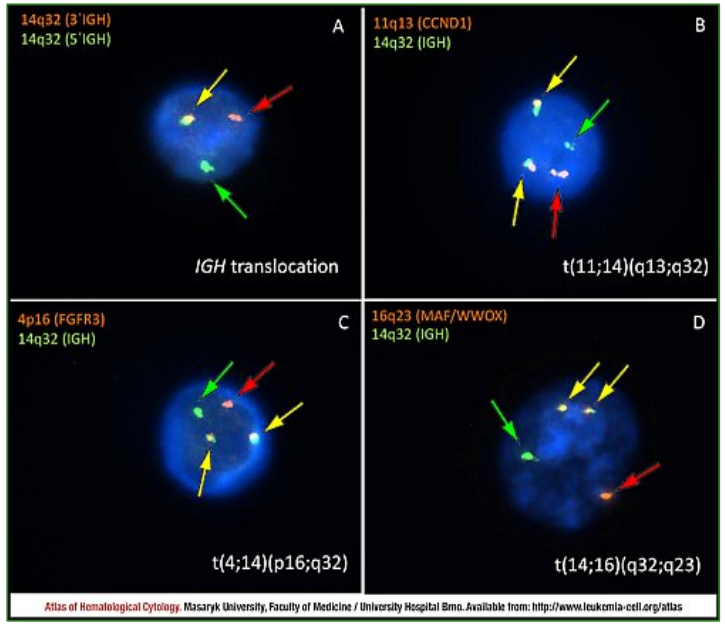


Advantages
- Whole-genome analysis
- Single-cell analysis

Disadvantages
- Requires mitotic cells
- Detects large abnormalities
- Resource intensive

FISH

Atlas of Hem Cytol - https://www.leukemia-cell.org/atlas/



Advantages
- High sensitivity
- Uses interphase cells
- Detects smaller abnormalities

Disadvantages
- Not whole-genome analysis (locus-specific)

Cytogenetic abnormalities of plasma cell neoplasms

Common abnormalities are classified by occurrence during progression of neoplasms

Abnormality	Classification of the International Myeloma Working Group
Primary	**Early events that lead to plasma cell clone; present in MGUS** • Translocations at immunoglobulin heavy chain locus (14q23) → oncogene dysregulation - 11q13 (Cyclin D1), 4p16 (FGFR), 6p21 (Cyclin D3), 16q23 (c-MAF), 20q11 (MAF-B) • Gains of chromosomes - Trisomy 3, 5, 7, 9, 11, 15, 17
Secondary	**Later events associated with malignant transformation and disease progression** • Chromosomal copy number changes → loss of tumor suppressor function • Other somatic mutations dysregulating cell growth and turnover - del(17p), gain(1q21), t(4;14), t(14;20), MYC translocations and del(1p)

Rajkumar SV, Dimopoulos MA, Palumbo A, et al. *Lancet Oncol.* 2014;15:e538-e548.

Prognostic classification

Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART)

Criteria	Standard risk	Intermediate risk	High risk
Cytogenetic abnormalities	Trisomies t(11;14) t(6;14) None	Gain(1q) t(4;14)	Del(17p) t(14;16) t(14;20)
Median overall survival	7-10 Years	5 Years	3 Years
Percentage of newly diagnosed patients	75%	10%	15%

Mikhael JR, Dingli D, Roy V, et al. *Mayo Clin Proc.* 2013;88:360-76

Clinical staging of plasma cell neoplasms

International Myeloma Working Group Revised International Staging System (R-ISS)

- Based on laboratory data
- Stratifies patients by risk
  - Progression-free survival
  - Response to stem cell transplantation

R-ISS Stage	Criteria
I	• Serum β2-microglobulin <3.5 mg/L • Serum albumin ≥3.5 g/dL • Standard-risk chromosomal abnormalities by interphase FISH • Normal lactate dehydrogenase (LDH)
II	• Not R-ISS stage I or III
III	• Serum β2-microglobulin ≥5.5 mg/L and either • High-risk chromosomal abnormalities by interphase FISH – OR – • High lactate dehydrogenase (LDH)

International Myeloma Foundation. International staging system (ISS) and revised ISS (R-ISS). Accessed Mar 24, 2023. https://www.myeloma.org/international-staging-system-iss-reivised-iss-r-iss

Assessment of minimal residual disease (MRD)

Several emerging methods

- International Myeloma Working Group definition of minimal residual disease (MRD):
  - Low levels of cancer cells in complete remission (CR) patients
  - Approximately 1 tumor cell in ≥100,000 normal bone marrow cells
- MRD-negativity is a positive prognostic marker in patients undergoing therapy
- Methods for assessment: aim is high analytical sensitivity for tumor cell markers
  - Next-generation flow cytometry (standardized 8-color protocol)
    - Markers: CD38, CD138, CD45, CD19, CD27, CD28, CD56, CD81, CD117, cytoplasmic Ig-κ, cytoplasmic Ig-λ, and β2-microglobulin
  - Next-generation sequencing
    - Clonal immunoglobulin heavy and light chain genes are targeted
  - Positron-emission tomography/computerized tomography (PET/CT)

Kumar S, Paiva B, Anderson KC, et al. *Lancet Oncol.* 2016;17:e328-e346

Summary

Laboratory methods for diagnosis and management of plasma cell neoplasms

- **Diagnosis**
  - Serum protein electrophoresis (SPEP)
  - Serum immunofixation by electrophoresis (SIFE)
  - Serum free light chain (SFLC) quantification and quantitative immunoglobulins
  - Urine protein electrophoresis (UPEP)
  - Urine immunofixation by electrophoresis (UIFE)
  - Bone marrow evaluation by Wright stain, histology, flow cytometry and cytogenetics
- **Prognosis**
  - Lactate dehydrogenase and beta-2 microglobulin
  - Molecular and cytogenetic analysis
- **Monitoring**
  - Serial SPEP, SFLC, UPEP
  - Minimal residual disease (MRD) testing by flow cytometry or molecular analysis

Emerging topics

Progress in diagnosis, prognosis, and therapy

- **Diagnosis:** Assessment of monoclonal immunoglobulins by mass spectrometry
  - Detection, quantification, and characterization of M-proteins
    - Analogous to protein electrophoresis and immunotyping (immunofixation or immunosubtraction)
  - Potentially more sensitive, specific, and practical to automate
- **Prognosis:** Progress in minimal residual disease testing
  - Standardization of more sensitive methods
  - Use of MRD-negativity as a criterion for approval of new therapies
- **Therapy:** Chimeric antigen receptor (CAR) T-cell therapy
  - Form of gene therapy
    - T cells isolated from patient and altered to express CAR against myeloma cells
    - Altered CAR T cells are infused back into the patient to attack myeloma cells

THANK YOU

Andrew N. Young, MD, PhD

Regional Medical Director, Southeast

Andrew.N.Young@QuestDiagnostics.com

Quest Diagnostics®

POWERING AFFORDABLE CARE SINCE 1967